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SEM Studies on Vessels in Ferns. 16. Pacific Tree Ferns (Blechnaceae, Cyatheaceae, Dicksoniaceae)[1]

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ABSTRACT: Scanning electron microscope (SEM) studies of tracheary elements of one species each of *Sadleria* (Blechnaceae), *Alsophila* (Cyatheaceae), *Cibotium*, and *Dicksonia* (Dicksoniaceae) showed that metaxylem of both roots and stems contains vessels with scalariform lateral wall pitting and scalariform perforation plates in which perforations are like lateral wall pits in size and shape. In Cyatheaceae and Dicksoniaceae, rhizome tracheary elements are short, contorted, with numerous facets. Several end wall facets of any given tracheary element in all genera studied can be perforation plates. At upper and lower ends of perforation plates, perforations have pit membrane remnants that contain porosities of various sizes, from large (nearly as big as the perforation) to extremely small (at the limit of resolution); the porosities are mostly circular in outline. No tracheids were observed with certainty. All tree ferns studied lack modifications of perforation plates like those of xeric ferns. The rhizome tracheary elements of Cyatheaceae are like those of Dicksoniaceae, but fusiform tracheary elements like those of many fern families occur in *Sadleria* (Blechnaceae); this correlates with the close grouping of Cyatheaceae with Dicksoniaceae in recent phylogenies that show Blechnaceae well removed from the tree fern families Cyatheaceae and Dicksoniaceae.

FERN TRACHEARY ELEMENTS can be studied with respect to ecology, systematics, and habit. Among xeric terrestrial ferns, we have studied three species of *Woodsia* (Carlquist et al. 1997, Carlquist and Schneider 1998*a*, Schneider and Carlquist 1998*a*) and one each of *Pteridium* (Carlquist and Schneider 1997*a*), *Astrolepis* (Carlquist and Schneider 1997*b*), and *Platyzoma* (Carlquist et al. 1999). All of these show modifications of the perforation plates (perforations wider axially to the long axis of the vessel than pits of lateral wall pitting), with the least degree of modification in the most mesic of the *Woodsia* species, *W. obtusa* (Spreg.) Torr. Presumably the specializations of the perforation plates permit flow of more water per unit time. Epiphytes would be expected to experience marked fluctuation in water availability that might, as in xeric terrestrial ferns, be correlated with perforation plates modified at least a little for rapidity of water conduction as compared with the patterns of lateral wall pitting. This proves to be true in rhizomatous epiphytic ferns such as *Phlebodium* (Schneider and Carlquist 1997) and *Microgramma* (Schneider and Carlquist 1998*b*), but not in rosette epiphytic ferns (Schneider and Carlquist in press). Rosette epiphytic ferns may have roots rather steadily in wet litter, whereas roots of rhizomatous epiphytic ferns are more exposed, potentially subjecting the ferns to greater fluctuation in water availability. Ferns with little fluctuation in water availability, such as Gleicheniaceae (Schneider and Carlquist 1998*c*) or Osmundaceae and Schizaeaceae (Carlquist and Schneider 1998*b*) have perforation plates with perforations like lateral wall pits in size. Perforation plates of such vessels cannot be identified or even suspected by mean of light microscopy, and scanning electron micros-

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copy (SEM) study is required to ascertain if end walls are composed of perforations or of pits. Tree ferns can all be characterized as mesic ferns, and thus if one hypothesizes specialized perforation plates in xeric ferns but not in mesic ferns, tree ferns should have vessels that correspond to those of Gleicheniaceae, Osmundaceae, and Schizaeaceae.

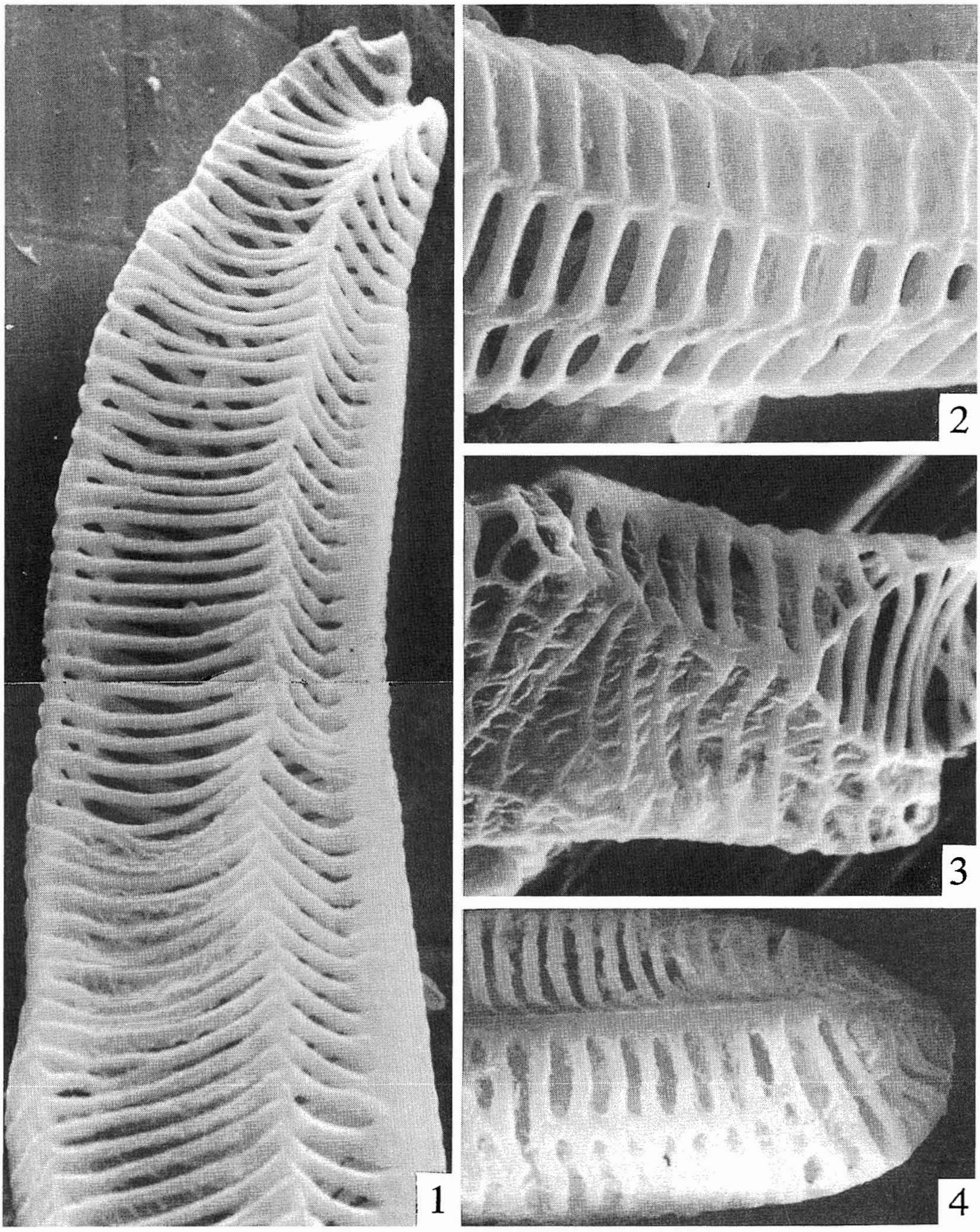
Tree ferns have a distinctive habit that invites comparisons between this habit and the nature of tracheary elements. One would not expect unusual tracheary element morphology so much in roots of tree ferns as in stem tracheary elements. The term "tree fern" is a misnomer not in terms of the form of the ferns so designated, but in terms of function. Unlike arboreal monocots, which have conductive elements of indefinite duration, stem tracheary elements at bases of tree ferns are dead, so that the adventitious roots must grow down from tracheary elements near the leaf rosette. Numerous connections between tracheary elements of roots and stems must be made to provide the leaf rosette with water. In addition, roots of tree ferns grow rapidly, as can be seen from observation of tree fern trunk surfaces, whereas the stems grow relatively slowly, and this differential may be related to tracheary element morphology. Although many features of tree fern vascularization have been subjected to analysis (Lucansky 1974*a,b*, Lucansky and White 1974), studies of tree fern tracheary elements by means of SEM have not been undertaken hitherto. Bierhorst (1960) included a number of ferns in his light microscopy studies of tracheary elements, but gave no details about tracheids of tree ferns.

Our SEM studies of fern tracheary elements have a systematic component; in addition to surveying ferns from divergent habitats and ferns of distinctive habits, we have attempted to cover a wide range of fern families, both eusporangiate and leptosporangiate. To be sure, our sampling has not been extensive because of the intensive work required to establish patterns of tracheary element structure by means of SEM. With this study, we have included the majority of leptosporangiate fern families in our survey. We have found that habitat and, to a lesser extent, habit are more important than phylogenetic position with relation to morphology of fern tracheary elements. Nevertheless, tree ferns offer an interesting case because of their phylogenetic positions relative to each other. In the phylogenies of Hasebe et al. (1995), Pryer et al. (1995), and Smith (1995), the families Cyatheaceae and Dicksoniaceae are very close to each other and on a branch including leptosporangiate ferns of intermediate specialization, whereas Blechnaceae are separated from Cyatheaceae and Dicksoniaceae. In all three phylogenetic studies, Blechnaceae occurs among more specialized fern families. If phylogenetic position is relevant to morphology of tracheary elements, one would expect that any differences observed would differentiate Blechnaceae on the one hand from Cyatheaceae and Dicksoniaceae on the other.

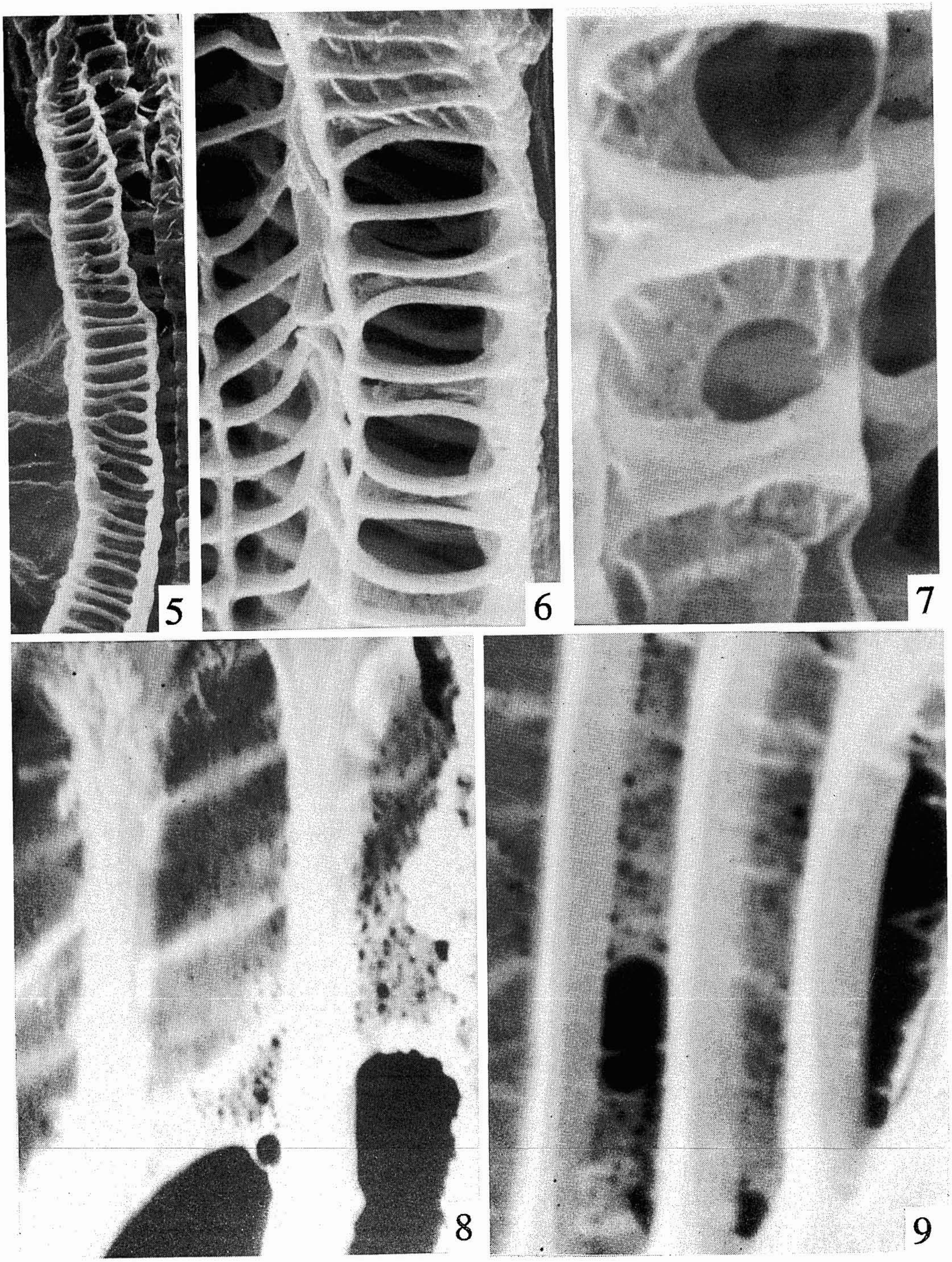
MATERIALS AND METHODS

Roots and stems were preserved in 50% ethyl alcohol. Because adventitious roots grow through stem tissue, care was taken to identify stem vascular tissue for preparations of stem tracheary elements. Macerations were prepared by means of Jeffrey's Fluid (Johansen 1940), stored in 50% alcohol, spread onto aluminum SEM mounts, air-dried, sputter-coated, and examined with an SEM (Bausch and Lomb Nanolab). In our earlier papers we discussed the distinctions we have used to distinguish between broken pit membranes and the rounded to oval holes and porosities that represent thin places where lysis has probably occurred during maturation of tracheary elements (Carlquist and Schneider 1997*a*, Carlquist et al. 1997). We developed criteria for distinguishing between artifacts and natural phenomena in those two studies by using both sectioned and macerated material and thus varying the treatment procedures.

The sources of the material are as follows: *Alsophila australis* R. Br. (Cyatheaceae), commercially grown specimen (rosette stage) purchased in Santa Barbara; *Cibotium splendens* (Gaud.) Kraj. ex Skottsberg (Dick-



FIGURES 1–4. SEM photographs of tracheary elements from roots (1–3) and stem (4) of *Sadleria cyatheoides.* 1. Tip of vessel element, showing two facets; the top 3/5 of the facet at left is a perforation plate, the lower 2/5 bears pits covered by pit membranes; the facet at right is a perforation plate. 2. Portion of a vessel element with three facets; the top facet bears pit membranes; the lower two facets lack pit membranes and are therefore portions of perforation plates. 3. A short vessel element, most of the length of which is shown; variously shaped pits covered with wrinkled pit membranes are seen throughout except at extreme upper left and at right, above, where pit membranes are absent and perforation plates therefore occur. 4. Tip of a tracheary element with scalariform pitting on all facets. Scale: Fig. 1 ×1700, Fig. 2 ×1800, Fig. 3 ×1835, Fig. 4 ×1600.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soniaceae), collected from the Lyon Arboretum (voucher at Lyon Arboretum, University of Hawai'i); *Dicksonia antarctica* Labill. (Dicksoniaceae), commercially grown specimen purchased in Santa Barbara; *Sadleria cyatheoides* Kaulf. (Blechnaceae), collected at the 14-mile marker on the Saddle Road, Hawai'i Island.

RESULTS

Blechnaceae

In roots of *Sadleria cyatheoides,* vessel elements are typically elongate and fusiform (Figures 1, 2), but a few short vessel elements (Figure 3) were observed. More than one end wall facet per vessel can bear a perforation plate (Figures 1, 2). The perforations are not different from lateral wall pits in size and shape (Figures 1–3). We did not observe porose pit membrane remnants in any perforations. The perforations seen were all clear of pit membranes. The pit membranes are striate (Figure 1), smooth (Figure 2), or wrinkled (Figure 3), but intact. All root tracheary elements illustrated are from metaxylem and have scalariform pitting or perforations (some pit and perforation shapes are modified in the short vessel element of Figure 3).

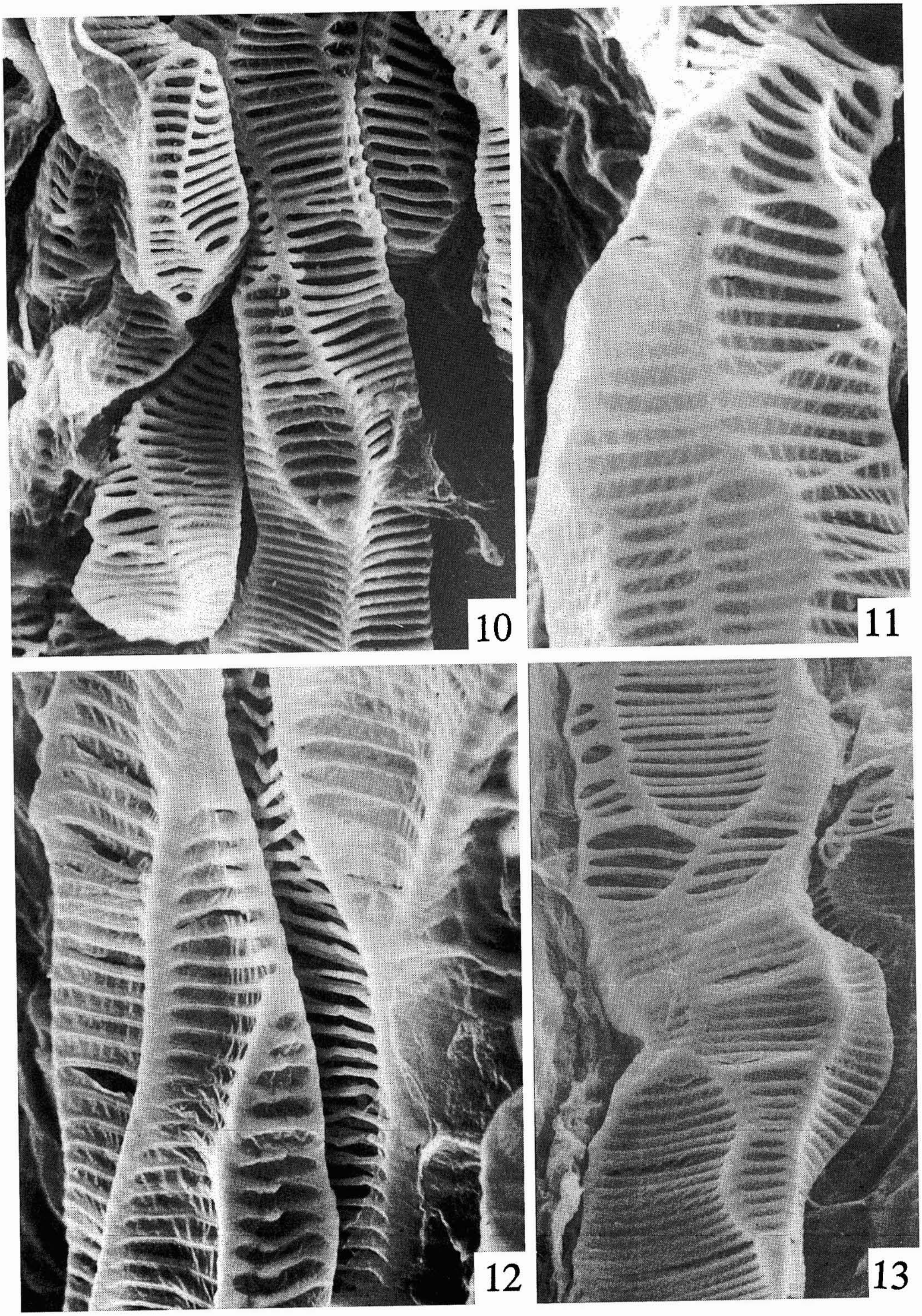
We were unable to isolate more than a few tracheary elements in preparations of rhizomes of *S. cyatheoides* because abundant parenchyma, fibers, and gelatinous contents of cells obscured the tracheary elements. We cannot affirm that the tracheary element portion in Figure 4 is typical; this element has scalariform pitting with intact pit membranes. Although stem (rhizome) vessel elements are abundant in ferns according to our studies, we cannot confirm the presence of vessels in stems of *S. cyatheoides.*

Cyatheaceae

Vessels are common in roots of *Alsophila australis* (Figures 5–7). Metaxylem vessels have scalariform perforation plates (Figures 5, 6). More than one facet of a vessel element may bear perforation plates (Figure 6, left element). The perforation plate in Figure 5 is typical, whereas Figure 6 shows some alternative configurations. Notably wide (in the direction of the element long axis) perforations are shown in Figure 6, upper left. In the element at right in Figure 6, there are wide perforations that alternate with narrow pits bearing intact pit membranes. This type of perforation plate construction was reported in *Phlebodium* (Schneider and Carlquist 1997) and *Anemia* (Carlquist and Schneider 1998*b*). Protoxylem tracheary elements are shown at low magnification in Figure 5, extreme right, and portions of protoxylem tracheary elements are shown enlarged in Figure 7. In Figure 7, each of the two perforations bears a pit membrane with a large central hole. The pit membrane remnants contain small oval to circular porosities of various diameters.

Stem tracheary elements of *Alsophila australis* are illustrated in Figures 8–13. In Figures 8 and 9, metaxylem pits located on lateral wall pitting near perforations show intermediacy in pit membrane presence. Large holes are present in some membranes

FIGURES 5–9. SEM photographs of vessels from root (5–7) and stem (8–9) of *Alsophila australis.* 5. Scalariform perforation plate (left), with portions of protoxylem tracheary elements at right; the bottom quarter of the element at left consists of lateral wall pitting. 6. Portions of two adjacent vessels; all facets of the vessel element at left are perforation plates; the facet at right shows five perforations, the bottom four of which are separated from each other by narrow pits with intact membranes; lateral wall pitting at top, above the perforations. 7. Portion of a vessel element perhaps transitional between protoxylem and metaxylem; a hole in each of the two pit membranes, top 2/3 of photograph, is surrounded by pit membrane portions in which minute pores are present. 8. Portion of perforation plate (axis of tracheary element oriented horizontally) showing two perforations in which pit membrane is probably naturally absent at bottom; the pit membrane remnants above in each perforation contain numerous small porosities of various sizes. 9. Portion of perforation plate (oriented as in Figure 8), in which four pit membranes are present; the one at far right is torn by processing; in the other pit membranes, porosities of various diameters plus a rather large natural hole are present (a tear is present below the hole). Scale: Fig. 5 ×1250, Fig. 6 ×3050, Fig. 7 ×9580, Fig. 8 ×8100, Fig. 9 ×9540.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(Figure 8, bottom; Figure 9, between lower left and center). These membranes also bear small pores of various sizes. Large numbers of these occur in the membranes of Figure 8. Smaller numbers of porosities are present in the membranes of Figure 9. With respect to shape and size, our sample of stem tracheary elements of *Alsophila australis* contained numerous elements with short facets (Figures 10, 11, 13). Some of these tracheary elements are short and contorted. Perforation plates are common in the tracheary elements we observed, although we cannot eliminate the possibility that tracheids in addition to vessel elements are present. Indeed, the tracheary element in Figure 11 shows facets composed of intact pit membranes, and no perforations were observed on this element (one cannot, of course, see facets concealed by being on back sides of the tracheary elements). All of the perforation plates observed are scalariform (e.g., Figures 10, 12), although some are distorted by contorted shapes of tracheary elements (Figure 13, top left).

Dicksoniaceae

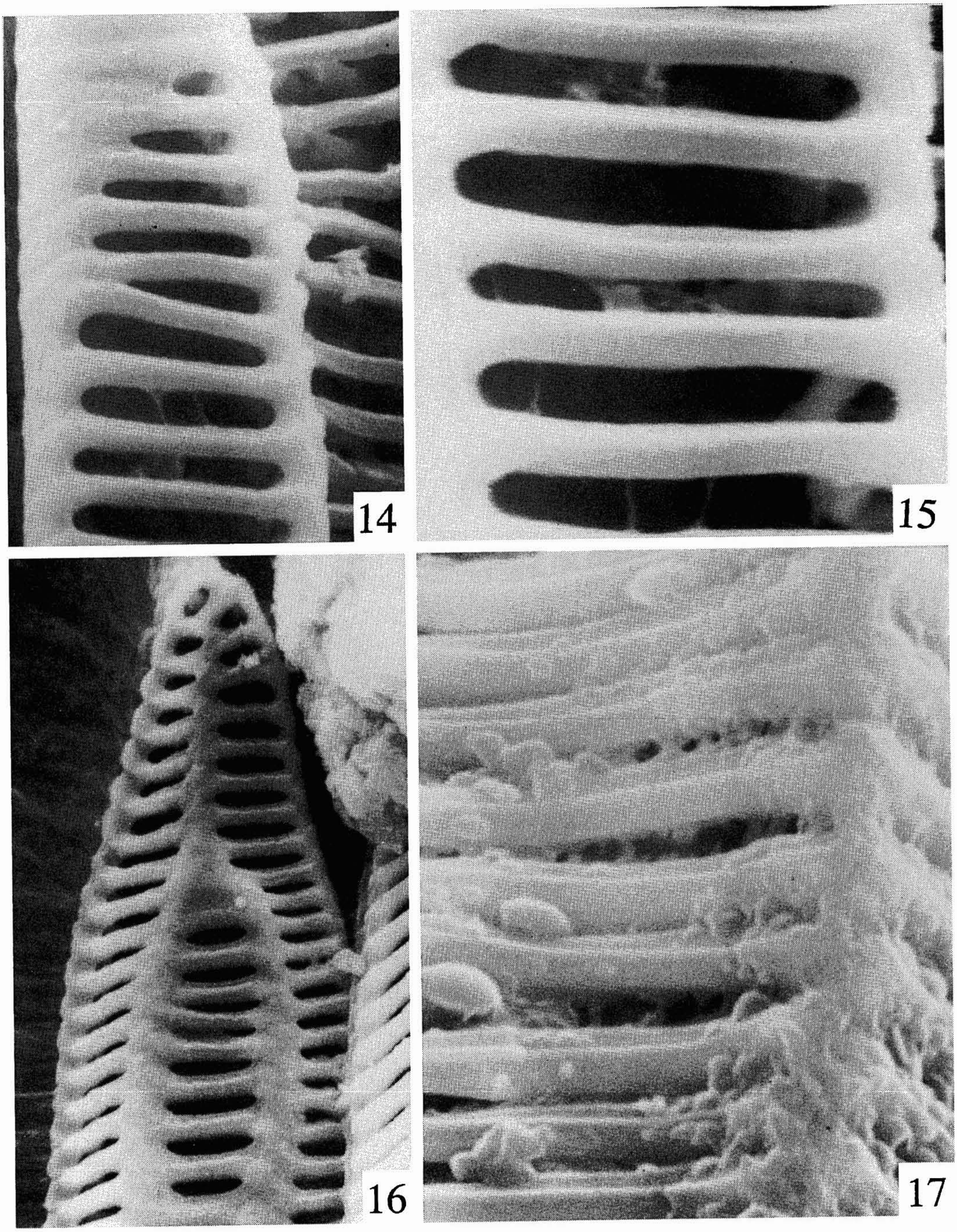
Tracheary elements in roots of *Cibotium splendens* (Figures 14, 15) show scalariform pitting and perforation plates. We have illustrated perforation plates in which there are pit membrane remnants in the perforations. Some of these take the form of threadlike primary wall strands (Figure 14, near bottom; Figure 15, top two perforations). A pit membrane that traverses most of a perforation is illustrated in Figure 15, center. This pit membrane remnant contains porosities of diverse sizes and shapes. All pits and perforations of metaxylem vessels are scalariform.

Rhizome metaxylem tracheary elements of *Cibotium splendens* (Figures 16, 17) have scalariform pitting and perforations. In Figure 16, the tip of a tracheary element is shown; all three facets are perforation plates. The tracheary element portion illustrated in Figure 17 is probably transitional between lateral wall pitting and perforations. Although some deposits cover some portions of this element, three pit membranes that clearly contain porosities of diverse sizes and shapes are present.

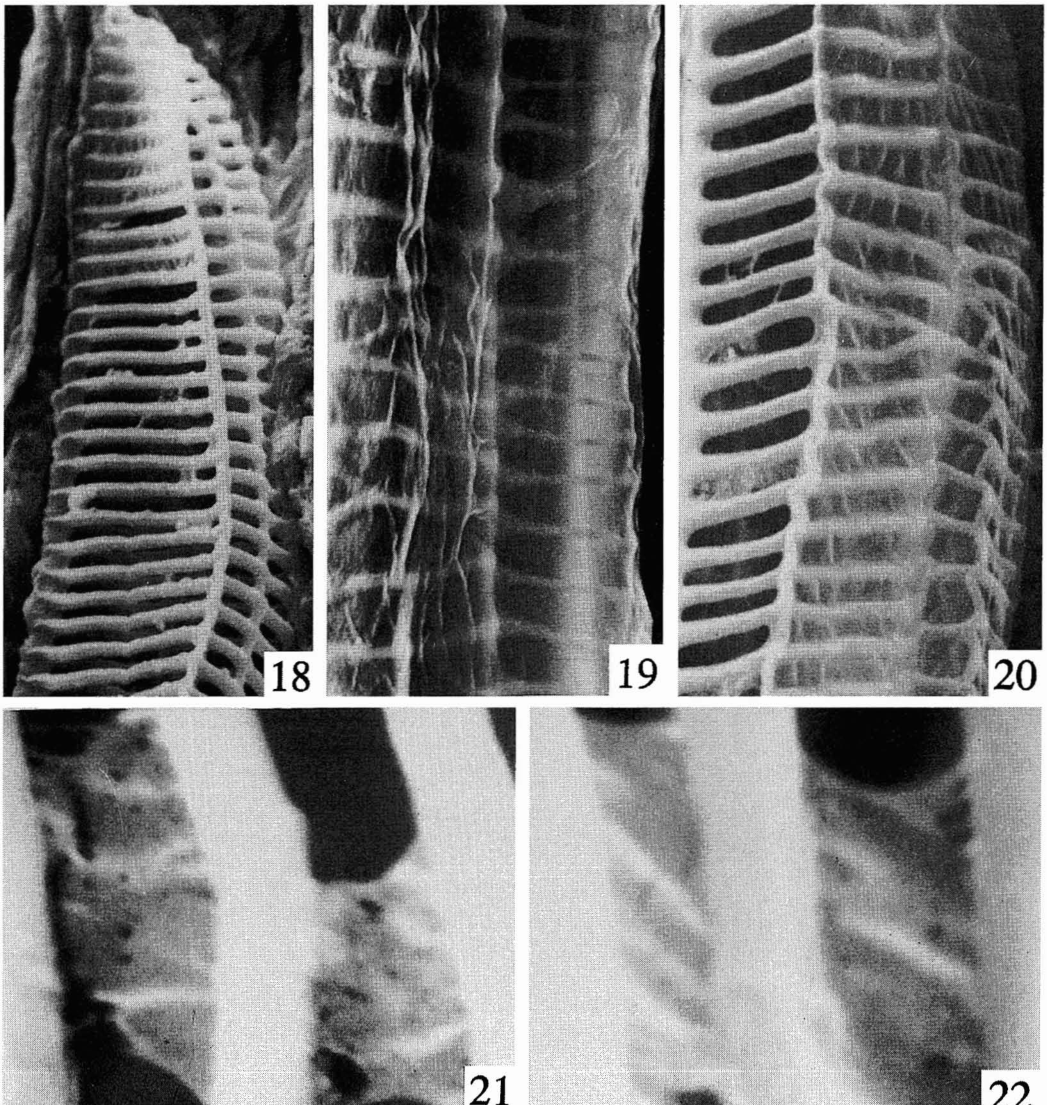
Figures 18–22 illustrate tracheary element portions from roots of *Dicksonia antarctica.* The metaxylem elements we observed have scalariform pitting and perforation plates (Figures 18, 20, 21, 22), whereas we saw only intact primary walls on protoxylem tracheary elements (Figure 19). Perforation plates of the metaxylem elements are not clearly defined in that they include pits with intact pit membranes (Figure 18; Figure 20, left). More than one end wall facet may bear perforations (Figure 18). Thin porose pit membrane remnants (Figures 21, 22) occur on pits adjacent to perforation plates (alternatively, these may be termed incipient perforations instead of pits). The porosities range widely in size, and these pit membrane remnants also contain large holes as wide (in an axial direction) as the perforation.

Rhizome metaxylem tracheary elements of *Dicksonia antarctica* (Figures 23–26) have scalariform pitting or perforations. The tracheary element tip shown at center in Figure 23 has a wide perforation plate at left, a very narrow perforation plate at center, and a facet bearing pits with intact pit membranes at right. The vessel element tip in Figure 24 has several perforation plates with no remnants of pit membranes, although narrow portions of facets shown at top left and bottom left have intact pit membranes. The tracheary element of Figure 25 has facets in which pit membrane remnants are present in some perforations, absent in others. A perfo-

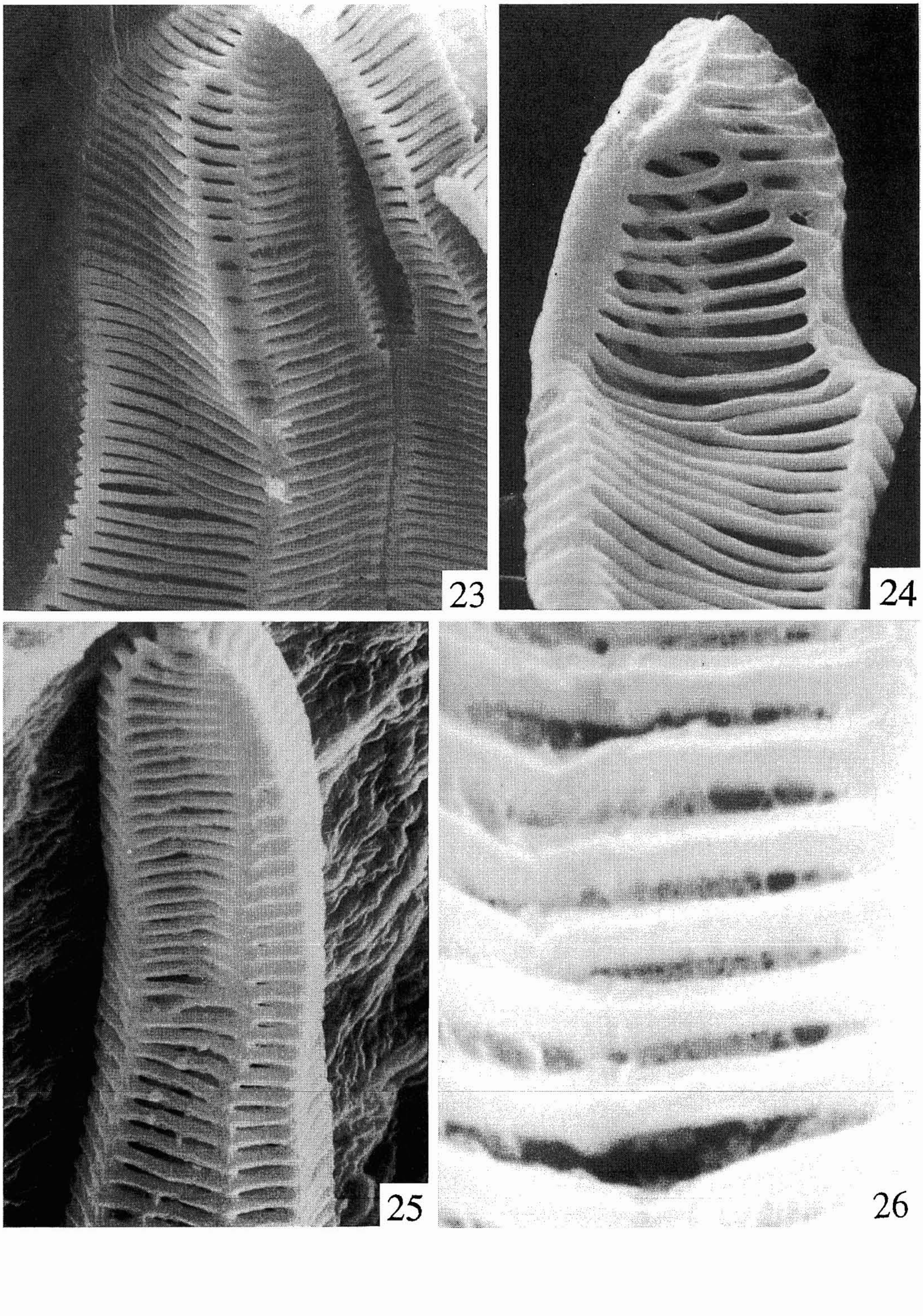
FIGURES 10–13. SEM photographs of rhizome tracheary elements of *Alsophila australis.* 10. Portion of a vascular strand with adjacent tracheary elements only slightly separated from each other; most of the facets illustrated lack pit membranes and are therefore perforation plates; many of the facets are relatively short and elliptical. 11. Near-terminal portion of tracheary element; facets are numerous, short, variously shaped, and all bear pit membranes. 12. Adjacent tracheary elements; all facets shown bear pit membranes except a narrow one to right of center, most of which is a perforation plate. 13. Contorted vessel element with numerous variously shaped facets; facets top center and left are perforation plates; pit membranes are present on all other facets. Scale: Fig. 10 ×820, Fig. 11 ×940, Fig. 12 ×1050, Fig. 13 ×660.



FIGURES 14–17. SEM photographs of tracheary elements of roots (14–15) and stems (16–17) of *Cibotium splendens.* 14. Portions of two adjacent perforation plates; the plate at left tapers and does not occupy the entire width of the wall; strands and pit membrane remnants are present in some perforations at left. 15. Perforations enlarged to show strands of primary wall material and, center, a pit membrane remnant with porosities. 16. Tip of a vessel element; pit membranes are entirely absent from all facets shown. 17. Portions of two facets; in three of the pit membranes of the left facet, porosities are present. Scale: Fig. 14 ×3020, Fig. 15 ×7130, Fig. 16 ×1070, Fig. 17 ×4900.



FIGURES 18–22. SEM photographs of tracheary elements of *Dicksonia antarctica* roots. 18. Metaxylem vessel element in which pit membranes are absent from all facets except at top of left facet. 19. Portions of two adjacent protoxylem tracheary elements; no perforations are present. 20. Portion of a tracheary element showing three facets; pit membranes are absent from most of the left facet. 21–22. Portions of two perforations (tracheary element axes oriented horizontally); in both of the pit membrane remnants in each figure, porosities of various sizes are present. Scale: Fig. 18 ×980, Fig. 19 ×2820, Fig. 20 ×3200, Fig. 21 ×9950, Fig. 22 ×11,100.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ration plate portion with variously thin pit membranes is illustrated in Figure 26. These pit membrane remnants are variously weblike and threadlike, and illustrate well intermediacy between the concepts designated by the terms pit and perforation.

DISCUSSION AND CONCLUSIONS

Our studies to date on fern tracheary elements have shown the presence of vessels in all genera and families we have studied. Presence of tracheids is difficult to establish, because one must be able to see a number of tracheary elements in which no perforation plates are visible on exposed surfaces of the elements. We have seen such tracheids in only one fern thus far, *Ceratopteris* (Carlquist and Schneider in press). The abundance of perforation plates in most ferns we have examined suggests that if tracheids are present, they are uncommon. The abundance of vessels in ferns may be related to the fact that tracheary elements occur in fascicles, so that numerous facets of one tracheary element touch similar facets of other tracheary elements, and on such facets, perforation plates often tend to occur. Several end wall facets of a fern vessel element are often perforation plates. The angiosperm model of vessels differs from this in that vessel elements form vertical series in which there is a single end wall at either end, and an end wall almost invariably bears a single perforation plate. The tree ferns examined in Blechnaceae, Cyatheaceae, and Dicksoniaceae agree with and enhance this picture of abundant vessel presence.

We have noted that at or near upper and lower tips of perforation plates (or even midway within a perforation plate) there may be porose pit membrane remnants. Although we did not see these in *Sadleria,* excellent examples of pit membranes bearing porosities were offered by tracheary elements of *Alsophila, Cibotium,* and *Dicksonia.* We have interpreted porose pit membrane remnants as incipient perforations.

Relevant with regard to perforation plates in the tree fern genera studied here is the similarity in secondary wall framework between facets that are perforation plates and those that bear pits with intact membranes. We have observed differences with respect to secondary wall framework between perforation plates and lateral wall pitting in xeric ferns; perforations are larger than pits in genera such as *Astrolepis, Pteridium,* and *Woodsia,* as cited in the introduction. On the other hand, ferns of mesic areas tend to have perforation plates identical to areas of lateral wall pitting, so that one can distinguish between the perforations and pits by means of SEM but not by means of light microscopy. The tree ferns studied here all agree with the mesic ferns in having this pattern.

Although all of the tree ferns we have studied have similarities in tracheary elements, our sampling is insufficient to determine if these cells offer features that would distinguish among the genera and families of tree ferns. The work of Bierhorst (1960) on fern tracheary element features suggests that distinctive features may characterize a few species or genera, but differences that would differentiate numerous genera within a family, etc., are not likely to be found. In other words, features of fern tracheary elements are not likely to be usable in key fashion to produce consistent and widely applicable systematic distinctions and similarities.

The short, often contorted tracheary elements of stems of *Alsophila australis* differ from tracheary elements of other ferns we have studied. The fact that these tracheary elements are so short and have so many short

FIGURES 23–26. SEM photographs of tracheary elements from *Dicksonia antarctica* stem. 23. Tip of one tracheary element and (at right) an edge of another; an exceptionally narrow facet, center of element at left, lacks pit membranes. 24. Tip of vessel element; pit membranes are absent on wide central facet but present on other facets. 25. Tip of vessel element; facet at left bears perforations or porose pit membranes. 26. Portion of tracheary element facet in which the pit membranes are markedly porose; the bottom pit membrane is torn. Scale: Fig. 23 ×1020, Fig. 24 ×2130, Fig. 25 ×1120, Fig. 26 ×7380.

facets may be related to the fact that the plant studied was relatively young, so that the stem was more like that of a rosette fern and unlike the elongate stem of a mature *Alsophila australis*. However we have not observed such short tracheids in other rosette ferns we have studied, such as species of *Woodsia* (Carlquist et al. 1997, Carlquist and Schneider 1998*a*, Schneider and Carlquist 1998*a*) or *Polystichum* (Schneider and Carlquist 1997). Further investigations will reveal whether this is a distinctive feature of *Alsophila australis* or has a distribution within ferns related to systematics or habit.

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